

Each human kidney contains about 1 million functional units that remove wastes from the blood, help control the body's fluid balance, and regulate electrolyte balance. New research in mice could help explain how kidneys generate the correct number of functional units, called nephrons, during development. (Left panel) Mice treated to have fewer nephron progenitor cells (bottom) during development have fewer nephrons at birth than normal mice (top)—a difference that persists into adulthood, even though overall kidney size is similar. (Right panel) Three dimensional renderings of the kidney internal structure during development show differences in size and extent of nephron branching between the normal (top) and progenitor-cell-limited (bottom) mice. As nephrons are not replaced after birth, having a lower nephron number at birth may cause kidney problems later in life. These new findings could contribute to efforts to understand and treat kidney disease.

Images courtesy of Dr. Frank Costantini, Columbia University Medical Center. Originally published in Cebrian et al, *Cell Rep* 7(1):127-137 2014. Photo copyright 2014 by Cebrian, et al. and adapted and reprinted under a Creative Commons Attribution License (<http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode>).

Kidney, Urologic, and Hematologic Diseases

Diseases of the kidneys, urologic system, and blood are among the most critical health problems in the United States. They afflict millions of Americans and their impact is felt across the lifespan. To improve our understanding of the causes of these diseases, and to identify potential new treatments for them, the NIDDK supports basic and clinical research studies of the kidney and urinary tract and disorders of the blood and blood-forming organs. The overall goal of the NIDDK's research programs is to increase our understanding of kidney, urologic, and hematologic diseases in order to enhance approaches to prevent and treat these serious conditions.

Normal, healthy kidneys filter about 200 quarts of blood each day, generating about two quarts of excess fluid, salts, and waste products that are excreted as urine. Loss of function of these organs, either for a short period of time or as a consequence of a gradual, long-term decline in kidney function, represents a life-threatening condition.

It has been estimated that more than 23 million Americans have impaired kidney function—also called chronic kidney disease (CKD).¹ CKD has two main causes: high blood pressure and diabetes. The increases in obesity and type 2 diabetes in the United States in recent years—especially among children and adolescents—have grave implications for the Nation's health, as young people with these conditions are likely to face serious health complications at an earlier age than people who historically have developed these conditions later in life.

One feature common to kidney diseases arising from varying causes is the deposition of fibrotic scar tissue in the kidney. Research supported by the NIDDK has enhanced our understanding of the origin of this scar tissue, how it can impair kidney function, and how it might be prevented or treated. As fibrosis is a common end point in several diseases, the NIDDK convened a meeting in 2014, “Targeting Fibrosis in Kidney, Bone

Marrow, and Urological Diseases,” to discuss ways to detect and measure fibrosis.

CKD, especially if undetected, can progress to irreversible kidney failure, a condition known as end-stage renal disease (ESRD). People with ESRD require dialysis or a kidney transplant to live. In 2012, over 636,000 patients received treatment for ESRD: nearly 450,000 received either hemodialysis or peritoneal dialysis and over 186,000 were living with a kidney transplant.² Racial and ethnic minority populations in the United States, particularly African Americans, Hispanic and Latino Americans, and American Indians and Alaska Natives, bear a disproportionate burden of CKD and ESRD. African Americans are nearly four times more likely to develop kidney failure than are non-Hispanic Whites.² American Indians and Alaska Natives and Hispanic and Latino Americans have twice the risk for kidney failure as do non-Hispanic Whites.² In recent years, scientists supported by the NIDDK have uncovered important genetic clues that may play a role in

¹ Levey AS, et al. *Ann Intern Med* 150: 604-612, 2009.

² U.S. Renal Data System, *USRDS 2014 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States*, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2014.

health disparities related to kidney disease susceptibility and progression in minority populations.

The NIDDK supports a significant body of research aimed at understanding the biology underlying CKD. The NIDDK's chronic renal diseases program supports basic and clinical research on kidney development and disease, including the causes of kidney disease, the underlying mechanisms leading to progression of kidney disease to ESRD, and the identification and testing of possible strategies to prevent development or halt progression of kidney disease. The NIDDK also supports studies of inherited diseases, such as polycystic kidney disease, congenital kidney disorders, and focal segmental glomerulosclerosis; and immune-related kidney diseases, such as IgA nephropathy and hemolytic uremic syndrome. The CKD Biomarkers Consortium (CKD BioCon) promotes the discovery and validation of novel biomarkers for CKD initiation, progression, and development of complications. A more complete understanding of biomarkers could allow physicians to detect kidney disease earlier and perhaps identify people at greater risk of progression, allowing them to tailor treatments to a specific individual.

The NIDDK's National Kidney Disease Education Program (NKDEP) is designed to raise awareness about the problem of kidney disease and steps that should be taken to treat CKD and prevent kidney failure. NKDEP represents a major educational outreach effort to patients, physicians, and the public. NKDEP also promotes the inclusion of estimates of kidney function as a part of routine blood testing and works to standardize measurements of protein in the urine, often a sign of underlying kidney disease.

Urologic diseases affect people of all ages, result in significant health care expenditures, and may lead to substantial disability and impaired quality of life. The NIDDK's urology research program supports basic and clinical research on the normal and abnormal development, structure, function, and injury repair of the genitourinary tract. Areas of interest include the causes of and treatments for urological diseases and disorders such as benign prostatic hyperplasia, urinary incontinence, and urinary tract infections. Other

disorders of the genitourinary tract, such as interstitial cystitis/painful bladder syndrome (IC/PBS)—also known as IC/bladder pain syndrome (BPS)—in women and men and chronic prostatitis/chronic pelvic pain syndrome in men, are also important components of the NIDDK's urology program. Additional areas of interest include research on treatments for kidney stones, such as shock-wave and laser lithotripsy to break up stones, and therapeutic approaches to inhibit their formation and growth.

IC/PBS is a debilitating, chronic, and painful urologic disorder. Based on a recent large national interview survey, it is estimated that 3.3 million (2.7 percent) U.S. women 18 years old or older have pelvic pain and other symptoms, such as urinary urgency or frequency, that are associated with IC/PBS.³ Using a community-based epidemiological survey, researchers have estimated that 1.6 million (1.3 percent) U.S. men ages 30 to 79 years old have persistent urologic symptoms, such as pain with bladder filling and/or pain relieved by bladder emptying, that are associated with painful bladder syndrome.⁴

NIDDK-supported basic and clinical research on IC/PBS is focused on elucidating the causes of these conditions, identifying “biomarkers” that will aid diagnosis, and improving treatment and interventions. Ongoing epidemiologic studies will help refine prevalence estimates and demographics. One example is the Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) research network, which supports research designed to uncover the underlying causes of IC/PBS and to characterize the disease profiles in patients; this network was recently renewed for an additional 5 years.

Urinary incontinence is conservatively estimated to affect 13 million Americans, most of them women.⁵ Many suffer in silence due to embarrassment and lack of knowledge about treatment options available.

³ Berry SH, et al. *J Urol* 186: 540-544, 2011.

⁴ Link CL, et al. *J Urol* 180: 599-606, 2008.

⁵ *Urological Diseases in America*. NIDDK, NIH Publication Number 07-5512, 2007.

NIDDK-supported studies over the past several years have helped to advance knowledge about the efficacy of surgical treatment of urinary incontinence, as well as provide new insights into non-surgical alternatives. As researchers continue to investigate treatment options, an equally important challenge is to improve strategies for assessing both the impact of urinary incontinence and other lower urinary tract symptoms in women and men and the effect of different diagnostic tools and interventions on patient outcomes; to address this challenge, the NIDDK launched and recently expanded the multi-site Symptoms of Lower Urinary Tract Dysfunction Research Network (LURN). The NIDDK is also leading new efforts to explore whether it may be possible to prevent symptom onset and/or progression, thereby improving health. The NIDDK, in conjunction with multiple other NIH Institutes and Centers, has launched a bladder health initiative to identify and characterize modifiable risk factors for lower urinary tract symptoms and urinary incontinence in women (see feature, “Paving the Way to Improved Bladder Health in Women”).

The NIDDK’s hematology research program uses a broad approach to enhance understanding of the normal and abnormal function of blood cells and the blood-forming system. Research efforts include studies of a number of blood diseases, including sickle cell disease, the thalassemias, aplastic anemia, iron deficiency anemia, hemolytic anemias, thrombocytopenia, and the anemia of inflammation and chronic disease. NIDDK-supported research has recently identified mutations that cause a rare anemia called X-linked sideroblastic anemia.

The NIDDK is also keenly interested in the basic biology of stem cells, including adult hematopoietic (blood) stem cells, which are needed for bone marrow transplants and may have broader application in gene therapy research.

MEASURING RISK OF KIDNEY DISEASE AND PROGRESSION

Genetic Variation and Chronic Kidney Disease Progression: African Americans who have chronic kidney disease (CKD) and two copies of common

variants in the *APOL1* gene are twice as likely to progress to kidney failure as those without these high-risk variants. Moreover, African Americans with the high-risk variants also tend to lose kidney function at twice the rate of those without the variant.

Previous studies have shown that African Americans with two copies of certain variants of the *APOL1* gene are at increased risk of developing kidney disease. Researchers attempted to further characterize the nature of this increased risk by analyzing data from people enrolled in two large studies of CKD: the Chronic Renal Insufficiency Cohort (CRIC) Study and the African American Study of Kidney Disease and Hypertension (AASK). The CRIC study is one of the largest and longest ongoing studies of CKD epidemiology in the United States; it is following both White and African American people with CKD, about half of whom also have diabetes. AASK was the largest and longest study of CKD in African Americans without diabetes whose CKD was attributed to high blood pressure. In the new analysis, the researchers found a correlation between the presence of high-risk variants of the *APOL1* gene and an increased risk of CKD progression among African Americans. This effect was seen regardless of whether patients maintained good blood pressure control or had diabetes.

This study builds on information learned over the past few years about how genetic factors can contribute to the increased risk of kidney disease in African Americans. These results have important implications for understanding the differences in kidney disease risk across populations. Moving forward, physicians may be able to make better choices about when to start screening for kidney disease and how to choose an appropriate therapy by identifying which patients have these gene variants and are therefore at increased risk of developing kidney disease and progressing to kidney failure.

Parsa A, Kao WHL, Xie D, ...Appel LJ; AASK Study Investigators, CRIC Study Investigators. APOL1 risk variants, race, and progression of chronic kidney disease. N Engl J Med 369: 2183-2196, 2013.

Approximating Kidney Function Through Urine

Analysis: The levels in urine of genetic material from a type of kidney cell correlate with loss of kidney function, according to a recent study. This finding offers the possibility of a new, noninvasive way to approximate the kidneys' filtering ability and monitor the progression of chronic kidney disease.

The podocyte is a cell that is a component of the glomerulus, the fundamental filtering apparatus in the kidney. It has been hypothesized that progressive loss of kidney function in many forms of kidney disease can be attributed, at least in part, to damage to the glomeruli and accompanying depletion of podocytes. As podocytes are lost, traces of them may be detectable in the urine. In the current study, investigators analyzed urine samples from over 350 adult and pediatric volunteers with kidney disease and compared them with urine samples from nearly 300 people without kidney disease. They observed a nearly 80-fold increase in podocyte-derived genetic material in patients who had glomerular disease (based on a previous biopsy) and who had progressive kidney disease. A second group of patients with Alport syndrome, a hereditary glomerular disease, exhibited a 23-fold increase in podocyte-derived genetic material. Interestingly, in people with autosomal dominant polycystic kidney disease, which does not feature glomerular injury, urine podocyte-derived genetic material was not increased. These observations suggest that the presence of podocyte genetic material in the urine may be a marker for glomerular injury and may provide a window into glomerular function.

These findings support the hypothesis that podocyte depletion is an important element of some forms of progressive kidney disease. Furthermore, measurement of urine levels of podocyte-derived genetic material may provide an easy, non-intrusive way to evaluate and monitor podocyte health in people with glomerular diseases.

Wickman L, Afshinnia F, Wang SQ, ...Wiggins RC. Urine podocyte mRNAs, proteinuria, and progression in human glomerular diseases. J Am Soc Nephrol 24: 2081-2095, 2013.

Genetic Factors in Kidney and Cardiovascular

Disease: Variants in the *APOL1* gene are associated with increased risk of kidney disease but not cardiovascular disease in African Americans with high blood pressure. Previous studies have shown that African Americans with two copies of certain variants of the *APOL1* gene are at increased risk of developing kidney disease from causes other than diabetes, but there have been conflicting data regarding the role of *APOL1* in cardiovascular disease.

Elevated blood pressure is relatively common in the U.S. population and is a risk factor for heart disease, stroke, and kidney disease. The Systolic Blood Pressure Intervention Trial (SPRINT) is testing whether reducing systolic blood pressure to a lower goal than currently recommended will reduce cardiovascular disease risk in people with high blood pressure but not diabetes. ("Systolic" refers to the higher of the two numbers in a blood pressure reading; it measures the pressure in the arteries when the heart beats. "Diastolic" refers to the lower of the two numbers and measures the blood pressure when the heart rests between beats).

Over 2,500 African American volunteers in the SPRINT study agreed to undergo genetic testing to allow researchers to examine their *APOL1* status as it related to their kidney function and risk of developing cardiovascular disease. Researchers found that study participants with two risk variants of the *APOL1* gene were more likely to have mild kidney disease, defined as diminished filtering capacity and/or protein in their urine, than people with a single risk variant or none. However, they were not more likely to have cardiovascular disease.

These data from the SPRINT study add to information learned over the past few years about the contribution of genetic factors to the increased risk of non-diabetic kidney disease in African Americans. The current investigation confirms that the presence of these variants is associated with mild kidney disease in this population but shows that they are not associated with cardiovascular disease. Although a key priority in treating high blood pressure is to reduce the risk of both

kidney and cardiovascular disease, more research is needed to identify the role played by *APOL1* variants in these two processes.

Langefeld CD, Divers J, Pajewski NM, ... Freedman BI. Apolipoprotein L1 gene variants associate with prevalent kidney but not prevalent cardiovascular disease in the Systolic Blood Pressure Intervention Trial. Kidney Int 2014 Jul 16. doi: 10.1038/ki.2014.254.

KIDNEY DISEASE TREATMENT

Origin of Cells Involved in Kidney Repair Following Injury: A recent study in mice suggests repair to the kidney's proximal tubules following injury is mediated by proliferation of existing mature cells rather than by activation of stem cells.

After acute injury in the kidney, such as that caused by the loss and sudden restoration of blood flow, cells may be damaged and less able to filter blood, and some die. New cells in the proximal tubule appear to replace the impaired cells and restore the tubules' function; however, the origin of these new cells has been the topic of much debate. Some evidence suggests that they are derived from existing, mature cells in the proximal tubule that multiply; other evidence points to a population of adult stem cells in the kidney as the source of these cells.

To address this question, researchers using a mouse model inserted a gene into the DNA of mature cells of a specific type that are only found in the proximal tubule of the kidney to label these cells and any cells derived from them. They reasoned that if repair following injury were accomplished by an expansion of the number of mature cells, there would be an increase in the number of labeled cells. Alternatively, if repair were mediated by stem cells (which would not have this label), there would be no increase in labeled cells. The results of the experiment showed an increase in the number of labeled cells in the mouse kidney after kidney injury, and that a larger number of labeled cells was seen in more severely injured kidneys. Similar findings were seen in mouse kidney tubule cells that

were grown in the laboratory. Interestingly, several genes associated with stem cells were "turned on" in the mature cells following injury.

This study provides evidence that repair following acute kidney injury occurs through a return of mature proximal tubule cells to a somewhat more stem cell-like state, followed by their proliferation.

Kusaba T, Lalli M, Kramann R, Kobayashi A, and Humphreys BD. Differentiated kidney epithelial cells repair injured proximal tubule. Proc Natl Acad Sci USA 111: 1527-1532, 2014.

Design Change May Improve Kidney Stone

Treatment: New research has shown that changes to a medical machine called a lithotripter may improve kidney stone treatment. Kidney stones are one of the most common disorders of the urinary tract. Kidney stones can form when substances in the urine—such as calcium, oxalate, and phosphorus—become highly concentrated. A small stone may pass on its own, causing little or no pain. A larger stone may get stuck along the lower urinary tract and can block the flow of urine, causing severe pain and/or bleeding. The lower urinary tract is primarily made up of the ureters (two tubes connecting the kidneys to the bladder), bladder, and urethra (tube that carries urine from the bladder to the outside). One treatment option for a person with a larger stone, or one that blocks urine flow and causes great pain, is noninvasive shock wave lithotripsy. In shock wave lithotripsy, the lithotripter generates shock waves that pass through the person's body to break the kidney stone into smaller pieces to pass more readily through the urinary tract. The lens component of the lithotripter serves to focus the shock wave at the selected target (stone). Although current lithotripters are more powerful and more reliable than previous iterations of the machine, their treatment effectiveness is reduced.

NIDDK-supported researchers have recently determined that the more powerful lithotripters shift the shock wave off-target, which contributes to efficiency loss. In addition, this same group of investigators made modifications to the lens while keeping all other

aspects of the lithotripter the same. By introducing a groove around the outer portion of the lens, the researchers were able to redirect the shock wave to its proper target when tested in an animal model. The newly designed lithotripter pulverized 89 percent of the stones to sufficient size for passage through the urinary tract compared to 54 percent of the stones by the current generation of lithotripter. Moreover, it is anticipated that the newly designed lithotripter will cause less damage to surrounding tissue. If future research shows similar benefits in people with kidney stones, this newly designed lens may be adaptable by other manufacturers to improve their lithotripters currently in medical practice.

Neisius A, Smith NB, Sankin G, ...Zhong P. Improving the lens design and performance of a contemporary electromagnetic shock wave lithotripter. Proc Natl Acad Sci USA 111:E1167-E1175, 2014.

Treatment with Two Antibiotics Dramatically Reduces Risk of Urinary Tract Infections in

Children with Vesicoureteral Reflux: Long-term use of a drug combination can reduce the risk of recurrent urinary tract infection by up to 80 percent in children with vesicoureteral reflux (VUR). In children with this condition, developmental abnormalities in one or both ureters—the tubes connecting the kidneys to the bladder—allow urine to flow back from the bladder into the ureters, and sometimes into the kidneys. As a result, children with VUR are more likely to have recurrent urinary tract infections (UTIs), which can increase their risk of kidney scarring and the potential for kidney failure.

For decades, doctors have treated children who have VUR with a small daily dose of the antibiotics trimethoprim and sulfamethoxazole (TMP/SMZ), often for years, with the hope of preventing recurrent UTIs and kidney damage. Although this approach seemed logical, there was no conclusive evidence that it provided long-term benefits. The Randomized Intervention for Children with Vesicoureteral Reflux (RIVUR) study examined the use of this drug combination in a well-defined population of over 600 young children with VUR whose ages ranged

from 2 months to 6 years over a 2-year period. RIVUR found that the risk of recurrent infection was reduced by 50 percent in children with VUR who received TMP/SMZ. Children with VUR and bladder and bowel dysfunction saw an even greater reduction, up to an 80 percent lower risk of recurrent infections.

While TMP/SMZ significantly reduced the incidence of recurrent UTIs during the trial, the number of children who developed kidney scarring did not decrease in the group receiving the antibiotics. The researchers suggest this may be due to parents' heightened vigilance for UTI symptoms and early treatment in the trial and because most of the children were enrolled after their first infection rather than after multiple infections, when more scarring might occur.

Further analysis of data from the RIVUR trial may provide insight into other factors that could reduce susceptibility to recurrent UTIs and kidney scarring. For now, though, the RIVUR study has demonstrated that treatment with TMP/SMZ offers the possibility of fewer infections for children with VUR, which may provide an opportunity for many of them to outgrow reflux as their bodies develop and mature.

The RIVUR Trial Investigators. Antimicrobial prophylaxis for children with vesicoureteral reflux. N Engl J Med 370: 2367-2376, 2014.

KIDNEY FORMATION AND FUNCTION

Disruption of Kidney Development Has Life-long

Consequences: Depletion of a subset of cells in the developing mouse kidney resulted in fewer filtering units, a deficit that persisted throughout life. This study provides important information regarding one of the factors that contributes to the kidneys' ability to remove waste products.

Nephrons are the basic functional unit of the kidney; they consist of various cells and structures that work together to filter waste products and excess fluid from the blood. Nephrons arise through an interaction between progenitor cells and ureteric buds as the kidneys

form. The researchers used genetic manipulation to generate mice in which they could selectively eliminate progenitor cells at a specific stage of development while leaving the surrounding cells unharmed, resulting in a 40 percent reduction in the number of progenitor cells. In response, there was a corresponding decrease in the rate at which the ureteric buds grew and branched. This adjustment allowed the developing kidney to maintain the proper ratio of progenitor cells to ureteric buds, but at the cost of sharply limiting the overall number of nephrons in the kidney.

There is no evidence for the growth of new nephrons in humans after birth, and nephrons that are lost due to disease or injury cannot be replaced. A fuller understanding of kidney development may provide clues as to possible strategies to grow new nephrons in kidneys, which could allow lost kidney function to be restored.

Cebrian C, Asai N, D'Agati V, and Costantini F. The number of fetal nephron progenitor cells limits ureteric branching and adult nephron endowment. Cell Reports 78: 127-137, 2014.

Chloride Concentrations Help Regulate Salt Metabolism by Influencing a Key Enzyme: A direct interaction between chloride and the enzyme WNK1 plays an important role in the kidneys' regulation of salt levels and blood pressure.

Cell-membrane-spanning co-transporter proteins regulate the levels of sodium, potassium, and chloride. In the kidney tubules, the return of these salts from the

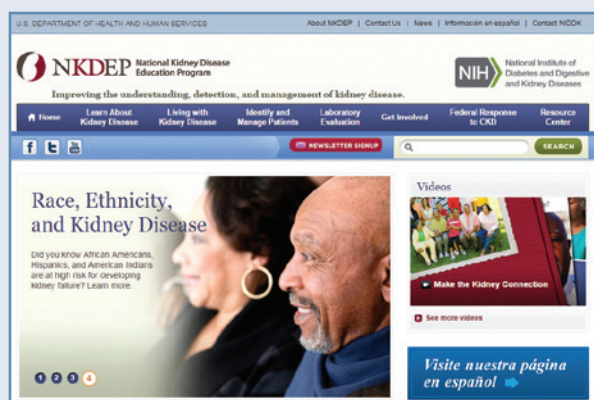
urine to the blood helps to maintain proper salt balance, fluid retention, and blood pressure. The activity of these co-transporters is closely associated with the levels of chloride inside of cells. Because of this correlation, researchers have hypothesized that these co-transporters might be under the control of a chloride-sensing mechanism. Researchers have now identified the regulatory enzyme WNK1 as one such regulator.

When chloride levels within the cell fall, WNK1 sets in motion a signaling cascade that leads to the activation of the sodium/potassium/chloride co-transporters and a corresponding increase in intracellular concentrations of these ions. Conversely, when chloride levels rise, WNK1's ability to activate the co-transporters is inhibited. Mutations to WNK1 that rendered it less able to bind chloride removed this inhibition, validating chloride as a modulator of WNK1 activity.

In humans, mutations in the WNK family of enzymes are associated with hyperaldosteronism II, an inherited disorder characterized by impaired salt secretion in the kidney that results in hypertension and elevated levels of potassium and chloride in the blood. A better understanding of the role of WNKs in salt metabolism may allow for the development of novel, targeted treatment strategies for hypertension and other diseases.

Piala AT, Moon TM, Akella R, He H, Cobb MH, and Goldsmith EJ. Chloride sensing by WNK1 involves inhibition of autophosphorylation. Sci Signal 7: ra41, 2014.

The NIDDK's National Kidney Disease Education Program Expands Its Outreach and Professional Education Initiatives



Educating those who are living with or at risk for chronic kidney disease (CKD) is a critical part of care. Providing straightforward, comprehensive information by a multidisciplinary team of health care professionals can encourage self-management and support informed decision-making, ultimately leading to improved outcomes.

In addition to a broad range of educational brochures and fact sheets, the NIDDK's National Kidney Disease Education Program (NKDEP) develops resources to help health care providers and educators present kidney health information to the public. NKDEP's newest resources include training programs for diabetes educators, community health workers, and pharmacists and a guide for primary care clinicians.

Providing kidney health resources for diabetes educators. Diabetes is the leading cause of CKD. Diabetes educators play a crucial role both in supporting early detection and slowing progression of CKD. NKDEP has developed a training program for diabetes educators, for which the American Association of Diabetes Educators provides continuing education credits. This program focuses on identification of kidney disease, slowing progression, addressing complications, and educating people with CKD.

Reaching out to the Hispanic community about kidney disease. NKDEP's *Riñones, Tesoros* ("Kidneys, Treasures") initiative aims to address the growing burden of kidney disease among Hispanics. The initiative's resources include Spanish-language educational materials, broadcast interviews, and an online newsletter, as well as a bilingual educational program and materials for use by community health workers.

Supporting CKD management in primary care. To help health care professionals manage CKD in their practices, NKDEP developed *Making Sense of CKD: A Concise Guide for Managing Chronic Kidney Disease in the Primary Care Setting*. The guide emphasizes the most important considerations for evaluating and managing CKD, including identifying and slowing disease progression among those at highest risk for progression to kidney failure.

Educating pharmacists about drug-induced acute kidney injury. Drug-induced acute kidney injury is common and preventable. To help address this problem, NKDEP has developed a training program for pharmacists on advising those at high risk for acute kidney injury about safe use of some commonly used medications. The University of Kentucky provides continuing education credit for this training.

Established in 2000, NKDEP aims to improve early detection of CKD, facilitate identification of patients at greatest risk for progression to kidney failure, promote evidence-based interventions to slow progression of CKD, and support the coordination of federal responses to CKD. To learn more about NKDEP and its resources, please visit www.nkdep.nih.gov

URINARY TRACT INFECTIONS: DIAGNOSIS AND HOST DEFENSES

Evaluating the Predictive Value of Urine Tests

for Urinary Tract Infections: Results from a new study could help refine approaches to diagnosing and treating uncomplicated urinary tract infections (UTIs) in healthy, premenopausal women. UTIs are very common and occur most often in women. The majority of UTIs are caused by the bacterium *Escherichia coli* (*E. coli*), but a number of other microbes can infect the bladder, as well. To help diagnose a woman who has UTI symptoms, a health care provider may ask her to provide a urine sample, which she collects into a sterile container while in the midst of voiding her bladder. Once collected, the urine can be tested for the presence of certain microbes—often by seeing what types and amounts of microbes will grow in a laboratory setting. However, these so-called “clean catch” midstream urine samples can be contaminated by microbes living near the opening through which urine leaves the body, potentially confusing the results. Also, there is some uncertainty about how well the values for microbial growth used for diagnosing UTIs from these samples actually reflect the presence or burden of UTI-causing microbes in the bladder itself.

To determine how well midstream void samples predict causative agents in UTIs, researchers recruited 202 healthy, premenopausal women with symptoms of acute UTI. They obtained two urine samples from each woman, one from the bladder (by using a catheter inserted into the bladder *via* the urethra, the tube that carries urine from the bladder to outside the body), the other from a midstream void, and then compared the presence and burden of microbes in these paired samples. The researchers found that UTI-causing microbes grew in 142 specimens of bladder urine and 157 specimens of voided urine. The majority (65 percent) of women with UTI symptoms had *E. coli* in their urine, and there was a strong correlation between voided and bladder urine, even at *E. coli* growth values below those currently used to diagnose UTI. In contrast, they found that presence in midstream urine samples did not accurately predict bladder infection by two other, commonly detected microbes thought to cause UTIs.

These and other results suggest that testing of midstream void samples is best used for detecting and diagnosing the most common cause of UTIs, *E. coli*. This new information can help guide health care providers who treat UTIs. In addition, the fact that bladder urine samples from over a quarter of women with UTI symptoms did not yield any microbes under standard laboratory growth conditions serves as a reminder that there may be other microbes not identified by the tests, and/or non-infectious conditions with the same symptoms as UTIs.

Hooton TM, Roberts PL, Cox ME, and Stapleton AE. Voided midstream urine culture and acute cystitis in premenopausal women. N Engl J Med 369: 1883-1891, 2013.

Kidney Cell Response to Urinary Tract Infection

Helps Halt Bacterial Growth: Researchers have identified a new bodily defense mechanism deployed in the fight against urinary tract infections (UTIs). UTIs are very common and affect more women than men. The leading cause of UTIs is exposure to uropathogenic *E. coli* bacteria, also referred to as UPEC. While antibiotics resolve many infections, recurrence is common, and antibiotic resistance is rising; a better understanding of the natural course of UTIs could help lead to new treatments. In addition to flushing bacteria and infected bladder cells out of the urinary tract through urination, the body employs innate defenses, such as production of antimicrobial molecules, to stymie UTIs. In a new study, scientists investigated a suspected defense molecule called lipocalin 2 (LCN2). It is known that LCN2 can arrest the growth of *E. coli* and certain other bacteria by limiting their access to the essential nutrient iron. The researchers found that levels of LCN2 were very high in the urine of patients suffering from UTIs and fell as the UTIs resolved. Through experiments in female mice, they confirmed that urine levels of LCN2 rose in response to infection with UPEC, and that mice lacking LCN2 cleared UTIs much more slowly. By tagging both mouse cells and UPEC with light-emitting molecules and other visually detectable markers, the researchers were able to track the primary source of LCN2 during an infection to cells in the kidney called α -intercalated cells. Mice lacking these kidney cells were less well able to

suppress UTIs. These specialized kidney cells help maintain the acid pH of urine; when mice were exposed to UPEC, these cells quickly acidified their urine even further, which appears to be yet another defense against bacterial infection. While the exact mechanism has yet to be determined, evidence from this study suggests that contact with molecules produced by UPEC and/or with the bacteria themselves stimulates these kidney cells both to produce the LCN2 defense molecule and to acidify the urine within hours of infection. Discovery of this acute response that effectively targets the most common cause of UTIs expands the role played by the kidney in innate defense against such infections, and could also provide new insight into why diseases that damage the kidney's α -intercalated cells leave patients more susceptible to UTIs.

Paragas N, Kulkarni R, Werth M...Barasch J. α -intercalated cells defend the urinary system from bacterial infection. J Clin Invest 124: 2963-2976, 2014.

INSIGHTS INTO INTERSTITIAL CYSTITIS/PAINFUL BLADDER SYNDROME

Brain Region Changes Associated with Symptoms in Women with Interstitial Cystitis: A new study suggests that enlargement of certain brain regions is associated with symptoms in women with interstitial cystitis. Interstitial cystitis (IC), also called IC/painful bladder syndrome (PBS), is a urologic chronic pelvic pain syndrome whose symptoms include urinary urgency, frequency, and pelvic pain. The causes and risk factors for IC are not well understood, and there is no fully effective treatment. Researchers in the Multidisciplinary Approach to the Study of Chronic

Pelvic Pain (MAPP) Research Network are using a variety of scientific approaches to better understand IC and similar pain syndromes and lay the groundwork for development of clinical interventions to prevent and treat these conditions. An important aspect of chronic pain studies is the emerging understanding that different parts of the brain may become altered in a way that augments and/or maintains the experience of pain. Network researchers used a sophisticated brain imaging technique to determine whether there are differences in the volume of gray matter—the core component of brain tissue—between women with IC and those without, and whether those differences align with symptoms. Comparing results from 33 women with IC and 33 women without IC, the team found several brain regions in which women with IC had a significantly greater volume of gray matter, three of which are involved in pain processing. Further, the scientists found that greater gray matter volume in one pain-processing brain region, called the right S1, was associated with greater symptoms of overall pain, urinary urgency, and anxiety reported by women with IC on the day of the brain scan. This is the first study to show regional brain differences between women with IC and healthy counterparts. Future studies may help researchers understand the relationship between altered brain regions and pain sensitivity in IC patients, as well as the impact of other variables, such as co-occurring pain conditions and gender, on these brain changes.

Kairys AE, Schmidt-Wilcke T, Puiu T...Harris RE. Increased brain gray matter in the primary somatosensory cortex is associated with increased pain and mood disturbance in interstitial cystitis/painful bladder syndrome patients. J Urol 193: 131-137, 2015.

Paving the Way to Improved Bladder Health in Women

Daily life can be full of endless possibilities and joys, from spending time with friends and family to pursuing one's own work and interests. Urine leakage, bladder infections and pain, and day to day management of bladder problems, as well their personal and fiscal costs, can hinder full participation in these activities and also potentially increase risk for other serious health conditions. The NIDDK is pursuing a new endeavor focused on prevention of conditions affecting the bladder and lower urinary tract in women, with the larger goal of improving overall health.

Framing the Problem: LUTS, Bladder Health, and Impact

A variety of problems can affect the bladder and the urethra, the tube that carries urine from the bladder to outside the body. These include urinary incontinence (UI), urinary tract infections (UTIs), overactive bladder, and interstitial cystitis/painful bladder syndrome (IC/PBS). Researchers and health care providers use the term lower urinary tract symptoms, or LUTS, as an umbrella term to include all symptoms associated with any type of lower urinary tract dysfunction or condition.

Women bear a disproportionate share of the burden of LUTS compared to men. Some LUTS conditions, such as UI, increase in prevalence with age, while others, such as UTIs, can strike in youth or young adulthood, and become recurrent. Thus, LUTS are important across the lifespan. For some conditions, such as IC/PBS, the risk factors for onset and progression are still under study; for others, some risk factors have been identified—for example, childbirth is among several factors known to increase a woman's risk of developing UI.

Many millions of Americans suffer from LUTS. The yearly costs of management and treatment have been estimated at over 20 billion dollars;^{1,2} this estimate does not include costs from lost productivity. Bladder conditions are also intertwined in complex

ways with other serious, chronic, and costly health conditions. For example, obesity is another risk factor for development of UI. However, women who develop bladder leakage may then reduce their physical activity, exacerbating risk of weight gain and potentially increasing risk for the metabolic disease, type 2 diabetes. Diabetes, in turn, increases risk for development of UTIs. LUTS also increase risk of depression, which can also exacerbate obesity and other conditions and contribute to reduced work productivity. In older women with UI, the potential for fractures and falls, including life-threatening hip fractures, also increases. The impact of LUTS is thus not restricted to bladder issues and symptoms, but is also felt in its negative effects on overall health.

An enormous challenge with LUTS is that much of what is known about underlying conditions comes from people who have sought treatment. Many women choose to tolerate these conditions for extended periods of time before seeking care. Reasons that have been given range from embarrassment and stigma to thinking that these conditions are a normal part of aging. In addition to extended suffering, later care-seeking limits some of the insights researchers and health care providers can gain about the causes, triggers, or inciting events for onset of symptoms.

Moreover, researchers and health care providers do not have a good handle on what is “normal,” *i.e.*, what is the baseline for a healthy bladder across the lifespan? Instead, much of the focus has been on helping girls and women who are symptomatic and who seek treatment to address their symptoms as best as possible, with therapies that include medication, behavioral modifications, biofeedback, and surgery. An additional complexity in LUTS is that symptoms do not always indicate dysfunction, dysfunction does not necessarily cause symptoms (at least initially), and the connection between healthy bladder habits and presence or absence of symptoms is unknown.

Striking a New Path: Prevention

Until recently, the primary research and health care focus for LUTS has been on treating the problem. Now, the NIDDK is spearheading an effort to expand the research picture to include prevention and bring it to the forefront. Several factors have led the NIDDK to pursue this new research direction: as with obesity, research has suggested that many LUTS risk factors may be modifiable, and prevention possible. Further, the enormous burden of LUTS, the relationship of LUTS to other diseases and conditions, and the escalating costs anticipated—especially with an aging U.S. population—strongly suggest the value of trying to prevent onset or worsening of these conditions. At the same time, there is a lot to learn about what constitutes bladder health and dysfunction, and what approaches may be feasible in prevention. Several other NIH Institutes and Offices with interests in this area are helping to develop this new program focus, including the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, the National Institute on Aging, the National Institute of Nursing Research, the NIH Office of Research on Women's Health, the NIH Office of Behavioral and Social Sciences Research, and the NIH Office of Disease Prevention.

To help inform this new effort, in 2013 and 2014 the NIDDK organized several meetings with a spectrum of experts from the NIH and the external scientific and health care communities to obtain diverse opinions on research needs in women's urologic health overall, and specifically in the area of prevention research. This series of meetings culminated in a large scientific workshop on May 3-4, 2014, "Path to Prevention of Lower Urinary Tract Symptoms (LUTS) in Women: Bladder Health." This meeting gathered researchers, health advocates, policy makers, NIH scientific staff, and health care providers to discuss gaps in what is known and to provide input on research questions, strategies, and future directions to take as the new program moves forward. In particular, the NIDDK was seeking input on:

- Risk factor assessment and prioritization in age groups across the lifespan

- Construction of a population survey to obtain information about what women and girls know and do about their bladder and LUTS, which would then be used to develop research ideas to improve bladder health in women and girls
- Stakeholder groups relevant to research on bladder health and their potential roles in research and implementation of findings
- Taking action for public health awareness and engagement—who should be involved (delivery and audience), what activities and messages should be pursued, and how and when this should be done
- Clinical prevention intervention studies and designs in bladder and pelvic health to consider for the future

Experts were drawn from across multiple fields—not just urology and urogynecology, but also pediatrics, epidemiology, prevention research, behavioral science, physical therapy, nursing, aging, patient education, and other disciplines. Participants contributed a wealth of information from a variety of perspectives, and identified a broad spectrum of areas in which we have insufficient knowledge to determine the ultimate impact on bladder or overall health, or which can be considered as possible points of intervention to consider in research—for example, voiding behaviors and habits, work and school environments, and health beliefs.³

The input the NIDDK received at this meeting helped inform the development of a Request for Applications (RFA) on "Prevention of Lower Urinary Tract Symptoms in Women: Bladder Health Clinical Centers (PLUS-CCs)" (RFA DK14-004, now published). The purpose of this initiative—the next key step in program development—is to form a clinical research consortium focused on developing an evidence base for normal or healthy bladder function and identifying behavioral and other risk factors for conditions associated with LUTS. It is expected that this information will provide a foundation for future, prevention-focused intervention studies for LUTS in girls and women. It is anticipated that awards to establish this consortium will be made in late Fiscal Year 2015.

Leveraging What We Know: Summit on Urinary Incontinence

At the same time as the NIDDK is expanding its women's urologic health research program to include and emphasize prevention, the Institute has led efforts to assess what has been learned from NIH-supported trials of treatments for UI—a highly prevalent LUTS condition—in order to benefit patients. In March 2014, the NIDDK hosted a highly productive meeting of investigators from several completed NIH-supported clinical trials of treatments for UI to understand better how the results of the trials have been adapted into practice; to identify key messages that could be transmitted to patients not only by urology care specialists, but more broadly by other health care providers; and to elicit opinions on unmet needs in UI research that could help stimulate future studies on identification, diagnosis, and treatment.⁴

Notably, the NIDDK also supports a Symptoms of Lower Urinary Tract Dysfunction Research Network (LURN). The goals for this cooperative research network are to develop tools for better measurement of patient experiences of LUTS and to better define the phenotypes of men and women with symptoms of lower urinary tract dysfunction—*i.e.*, to determine what anatomical, physiological and psychological characteristics these individuals have that contribute to underlying functional changes that accompany LUTS. Characterization of patients with LUTS will enable the researchers to understand the underlying pathophysiology and such information could help identify specific subgroups of people suffering with LUTS by virtue of shared characteristics and thereby help to refine diagnoses and improve treatments. Currently, investigators at six sites are working

on developing self-reported measures for LUTS and ways to assess other, non-urologic factors contributing to LUTS. Parallel efforts are ongoing to establish a large cohort of people with LUTS and to conduct neuroimaging studies and sensory testing in people with urinary urgency, frequency, and incontinence.

Looking to the Future

The new focus for the women's urologic health research program at the NIDDK is a long-term effort to cover gaps in knowledge, move toward primary and secondary prevention of LUTS in women, and develop evidence-based public health messages about bladder health. While the new efforts are targeted to women due to the disproportionate burden of LUTS conditions on women, LUTS do affect both men and women. It is anticipated that, as more is learned, prevention research efforts will expand to include LUTS in men.

¹ Foxman B. *Epidemiology of urinary tract infections: incidence, morbidity, and economic costs.* *Am J Med* 113 Suppl 1A:5S-13S, 2002.

² Hu TW, Wagner TH, Bentkover JD, Leblanc K, Zhou SZ, and Hunt T. *Costs of urinary incontinence and overactive bladder in the United States: a comparative study.* *Urology* 63: 461-465, 2004.

³ A summary of the "Path to Prevention" meeting is available on the NIDDK website at www.niddk.nih.gov/news/events-calendar/Pages/LUTS.aspx#tab-minutes

⁴ A summary of the "Summit on Urinary Incontinence Clinical Research in Women" is available on the NIDDK website at www.niddk.nih.gov/news/events-calendar/Pages/summit-urinary-incontinence-clinical-research-women.aspx#tab-minutes

UNDERSTANDING AND TREATING HEMATOLOGICAL DISEASES

Newly Identified Mutations Cause Rare Anemia:

Recent research has identified previously unknown mutations in the *ALAS2* gene that cause X-linked sideroblastic anemia. X-linked sideroblastic anemia is an inherited disorder that prevents developing red blood cells (erythroblasts) from making enough hemoglobin, which is the protein that carries oxygen in the blood. Approximately two-thirds of X-linked sideroblastic anemia cases are due to mutations in the *ALAS2* gene. The known *ALAS2* gene mutations result in a change in an enzyme called erythroid ALA-synthase, which plays a critical role in the production of heme (a component of the hemoglobin protein). Thus, *ALAS2* mutations impair the activity of erythroid ALA-synthase, disrupting normal heme production and preventing erythroblasts from making enough hemoglobin. When not incorporated into heme, iron builds up in the erythroblasts in the bone marrow, and these abnormally iron-loaded cells are called “ring” sideroblasts. The symptoms of X-linked sideroblastic anemia result from a combination of reduced hemoglobin and the abnormal excess of iron. Ranging from mild to severe, the common features include fatigue, dizziness, a rapid heartbeat, pale skin, and an enlarged liver and spleen. Over time, severe medical problems such as heart disease and liver damage can result from the buildup of excess iron in these organs.

While studying the DNA from five families having a preponderance of X-linked sideroblastic anemia, investigators identified previously unknown mutations in the *ALAS2* gene. These mutations were discovered in a part of the gene called the enhancer—an element that helps “turn on” the *ALAS2* gene. Specifically, any of several changes in the enhancer sequence were sufficient to diminish *ALAS2* gene activation. This study’s characterization of a new set of mutations may help diagnose those having X-linked sideroblastic anemia, who can then be treated.

Campagna DR, de Bie CI, Schmitz-Abe K, ...Fleming MD. X-linked sideroblastic anemia due to ALAS2 intron

1 enhancer element GATA-binding site mutations. Am J Hematol 89: 315-319, 2014.

Potential New Treatment Option for Adults with

Sickle Cell Disease: A new blood stem-cell transplant regimen effectively reversed sickle cell disease in 26 of 30 adult participants and allowed them to achieve stable mixed donor chimerism, a condition in which a person has two genetically distinct cell types in the blood. Also of importance, 15 of the 30 adults were able to stop taking immunosuppressant medications 1 year after transplantation. In sickle cell disease, sickle-shaped red blood cells block blood flow. The blockage can cause severe pain, organ damage, and stroke. Mature red blood cells arise from stem cells. Transplantation of blood stem cells, obtained from bone marrow or another source such as umbilical cord blood, has been used to cure children with severe disease. However, the medical procedures used for preparing patients for transplantation have thus far been too toxic to be used in adults.

In this new regimen, instead of using chemotherapy to destroy the person’s bone marrow before infusing donor stem cells—as in the standard, prohibitively toxic procedure—the researchers used a low dose of radiation combined with two immunosuppressive drugs. This type of procedure is referred to as “non-myeloablative,” meaning that it does not destroy a person’s own bone marrow. Rather, it is thought to create “space” for the donor stem cells to successfully engraft. After undergoing the non-myeloablative procedure, the participants, who all had severe sickle cell disease, were infused with peripheral blood stem cells from healthy sibling donors. The researchers reported that the partial stem cell transplantation regimen effectively reversed sickle cell disease in the majority of adult participants, and half were able to stop immunosuppressant medications. These medications are typically given to transplant recipients to prevent the immune system from rejecting the transplanted donor cells and to prevent donor cells from attacking the recipient (graft-versus-host disease). However, immunosuppressant medications reduce immune system strength and can cause serious side effects such as infection and joint swelling. The researchers

reported that no graft-versus-host disease has been detected in patients after stopping immunosuppression medications at a median follow-up of 3.4 years.

This study represents an important advance to make a potentially transformative treatment available to a wider range of people, especially those who could not tolerate a standard stem cell transplant or long-term use of immunosuppressant medications.

Hsieh MM, Fitzhugh CD, Weitzel RP, ...Tisdale JF. Nonmyeloablative HLA-matched sibling allogeneic hematopoietic stem cell transplantation for severe sickle cell phenotype. JAMA 312: 48-56, 2014.

ADVANCES IN BLOOD CELL BIOLOGY

Gaining Insight into Blood Stem Cell Generation:

A recent study has illuminated the importance of a cellular protein called GATA2 in the generation and survival of blood (hematopoietic) stem cells in mice. Hematopoietic stem cells (HSCs) give rise to all the types of blood cells, including red blood cells, but the molecular mechanisms regulating the generative process are not completely understood. In contrast to HSCs, hematopoietic progenitor cells (HPCs) are relatively immature cells that are precursors to a fully mature (differentiated) cell of the same tissue type but that have only a limited capacity to differentiate into more than one cell type as HSCs do. As cells and tissues develop in the embryo, the process by which they take on specific characteristics involves cellular proteins called transcription factors, which regulate whether genes are “turned on” or “turned off.” Consistent with a potential role in HSC generation, the transcription factor GATA2 is known to be present in cells implicated in the formation of hematopoietic tissue in the developing mouse embryo, but its function in the generation of HSCs is unknown.

Using a “conditional knockout” strategy, investigators sought to examine what, if any, role GATA2 plays in HSC generation. Through this genetic engineering approach, scientists can produce mice that lack a specific gene only under certain conditions—a

so-called conditional knockout. This approach contributes valuable information about the normal function of a gene and its encoded product, such as GATA2, by allowing scientists to observe the consequences of its absence at specific times or developmental stages, and/or in particular tissues. The collective findings from this study indicate that GATA2 is required for HSC generation and HPC formation during embryonic development. In addition, GATA2 is required for survival of HSCs and HPCs in the developing embryo. Thus, these results highlight a unique role for GATA2 function in mouse embryonic hematopoiesis and should inform future regenerative approaches designed to treat blood diseases.

de Pater E, Kaimakis P, Vink CS, ...Dzierzak E. Gata2 is required for HSC generation and survival. J Exp Med 210: 2843-2850, 2013.

New Insight into the Mouse Blood Stem Cell

Aging Process: Recent research identified changes in blood (hematopoietic) stem cells (HSCs) that may contribute to age-associated loss of function. The HSC, a type of adult stem cell, holds great promise for future biomedical applications because of its ability to self-renew and develop into any kind of blood cell. However, previous research has shown that as the HSC ages, its capacity to develop into different types of blood cells diminishes. The mechanism(s) responsible for this change is not well defined.

New research has provided insight into the HSC’s aging process by systematically evaluating the gene expression (whether genes are turned “off” or “on”) in cells from both young and old male mice. For this study, the researchers used several techniques, including a process called transcriptome analysis, to determine the extent to which genes are on or off depending on the age of the HSCs. They also studied chemical modifications along the genome that affect gene expression. The genome is made up of DNA, a long, winding molecule that contains the instructions, in the form of genes, needed to build and maintain cells. For these instructions to be carried out, DNA must be transcribed into corresponding molecules of RNA, referred to as transcripts. A transcriptome is a collection of all the transcripts present in a given cell.

Often researchers can count the number of different types of transcripts in the transcriptome to determine the level of activity of different genes, also called gene expression, in a certain cell or tissue type. In humans and other multi-cell creatures, nearly every cell contains the same genes, but different cells show different patterns of gene expression. These differences in gene expression are responsible for the many different properties and behaviors of various cells and tissues as they experience health, normal aging, and disease. Cells also make various modifications along the genome, such as adding chemical “markers” to their DNA. Called “epigenetic” changes, these modifications affect gene expression.

A new analysis of mouse HSC transcriptomes and various epigenetic markers identified many genes that were expressed differently by old and young mice. Researchers found, for instance, that genes regulated by a growth factor called TGF- β showed differences in expression between young and old HSC cells. This finding suggests that there is less signaling by TGF- β in older cells. Previous research has shown that TGF- β helps control the growth and proliferation of cells, the process by which cells mature to carry out specific functions (differentiation), cell movement, and the self-destruction of cells. In addition, the researchers confirmed and extended previous studies by identifying epigenetic changes in young versus old HSCs that are consistent with the aging HSCs’ inability to develop into other types of blood cells.

Taken together, this study provides a comprehensive analysis of the genomic properties of young and old mouse HSCs and suggests how changes in the stem cell during aging promotes self-renewal and hinders HSCs’ ability to transition into other types of blood cells. This research provides new insight into the aging process and may be the basis for future treatments for aging-related disorders.

Sun D, Luo M, Jeong M, ... Goodell MA. *Epigenomic profiling of young and aged HSCs reveals concerted changes during aging that reinforce self-renewal.* *Cell Stem Cell* 14: 673-688, 2014.

Getting Heme into Hemoglobin: A recent investigation determined that a gene called *Tmem14c* is required for heme production within the mouse red blood cell. Heme is an iron-containing molecule that is important for many biological processes. Heme combines with globin proteins to form hemoglobin, which carries oxygen in red blood cells from the lungs to the rest of the body. Notably, there is virtually no “free” heme in the human body due to the potent heme-scavenging system in the blood. Within cells, heme levels are maintained by a balance of heme production, degradation, and distribution. The mechanisms of heme production and degradation have been intensively studied over several decades. However, the critical steps and location within the red blood cell where heme production takes place are largely unknown.

Building on their previous finding that initially identified the *Tmem14c* gene as having a role in heme production in the mitochondrial compartment of the red blood cell, researchers mechanistically dissected the functional role of this gene using biochemical, molecular biology, cell biology, pharmacologic, and genetic methods. The *Tmem14c* gene was found to be “turned on” in mouse embryonic tissues such as the yolk sac, liver, bone marrow, liver, spleen, and blood vessels—yolk sac, liver, and bone marrow play a role in red blood cell production in the embryo. The investigators also reported that the TMEM14C protein was produced in embryonic liver. To learn more about its possible function, the researchers determined that the protein is found associated with mitochondria, the home of cellular energy production. The researchers then examined blood cells derived from mouse embryonic stem cells that had been modified to no longer contain a functional *Tmem14c* gene. The resulting *Tmem14c* deficiency caused a decrease in the percentage of hemoglobin-containing red blood cells. In mice, *Tmem14c* deficiency (resulting from the combination of both copies of the *Tmem14c* gene being non-functional) was found to be lethal in at the embryonic stage, evidence that this gene is essential for normal development. In contrast, mice with one

copy of the non-functional gene were viable and fertile and had normal levels of red blood cells.

To examine its potential role in heme production, the ability of embryonic liver tissue to produce heme was assessed in the absence or presence of a functional *Tmem14c* gene. The investigators reported a buildup of pre-heme molecules in the absence but not presence of *Tmem14c*—indicating that a functioning gene is required for complete heme production.

This study provides valuable insight into the role of *Tmem14c* in heme production and hemoglobin-containing red blood cells. Future studies may determine whether the human version of this gene contributes to fewer red blood cells than normal (anemia) in people with various blood disorders.

Yien YY, Robledo RF, Schultz LJ, ...Paw BH. TMEM14C is required for erythroid mitochondrial heme metabolism. J Clin Invest 124: 4294-4304, 2014.

STORY OF DISCOVERY

FGF-23 Steps into the Spotlight as a Key Player in Phosphate Metabolism and Kidney Disease

While the kidneys are perhaps best known for their role in cleansing the blood, another one of their important functions is to regulate the levels of various salts and minerals in the blood. One of the consequences of chronic kidney disease (CKD) is that, in addition to a diminished ability to filter waste, the kidneys are less well able to maintain the balance of salts and minerals, which can in turn have wide-ranging impacts on overall health. One important pathway under the influence of the kidney involves the regulation of phosphate. A recently characterized member of the family of fibroblast growth factors (FGFs), FGF-23, appears to play an important role in regulating the metabolism of phosphate, and may play a previously unappreciated role in the initiation and progression of CKD. Evidence supporting this hypothesis includes data from studies of animals, individuals with CKD and kidney failure, and people with acute kidney injury, suggesting that this factor may represent a broadly acting regulator of phosphate metabolism that plays a key role in kidney function.

The Kidneys and Calcium and Phosphate Metabolism

Proper kidney function is essential for life; people whose kidneys have failed must undergo dialysis or receive a kidney transplant to survive. The supply of donor organs is much smaller than the need for them, so most people with kidney failure rely on dialysis to survive. While dialysis provides a life-saving form of kidney replacement for people whose kidneys have failed, most people with CKD will die before they progress to kidney failure, with cardiovascular disease the most common cause of death.

In addition to its well-known and critical role in waste removal, the kidney plays an important role in the regulation of the balance of calcium and phosphate, two elements that are key to maintaining normal bone metabolism, cardiovascular function, and many cellular signaling processes. Calcium is the most abundant mineral in the human body. Ninety-nine percent of it is found in bone, much of it bound with phosphate; the remaining 1 percent circulates in the blood, where it plays an important role in metabolism. While approximately 10 percent of a person's bone mass is degraded and rebuilt each year, circulating levels of calcium and phosphate are held relatively constant through a complex regulatory system.

An early sign of impaired kidney function is often a small decrease in levels of calcium circulating in the blood coupled with a small increase in circulating phosphate. In response to these changes, the parathyroid glands (located in the neck) secrete parathyroid hormone (PTH). This hormone acts on the bones, kidneys, and gut to produce an increase in circulating calcium and a decrease in circulating phosphate. As CKD progresses, calcium and phosphate levels continue to skew, and PTH levels rise further in an attempt to restore balance of these two important elements. Unfortunately, chronically elevated levels of PTH can result in devastating bone loss as the body turns to the skeleton as a source of calcium. This condition led to the concept in the early 1970s of the so-called “trade-off hypothesis,” which proposed that a ratcheting-up of PTH in people with CKD, and its attendant consequences, was the “trade-off” the body made as it tried to maintain

STORY OF DISCOVERY

close-to-normal mineral balance. This hypothesis grew out of research supported by the NIH's National Institute of Arthritis, Metabolism, and Digestive Diseases, which in 1986 would become the National Institute of Diabetes and Digestive and Kidney Diseases and the National Institute of Arthritis and Musculoskeletal and Skin Diseases.

Although modern therapies can address the problem of bone loss in people with CKD through dietary modification and drugs, there is significant evidence that long-term exposure to elevated levels of phosphate in the blood is in and of itself a risk factor for cardiovascular disease in these individuals. These risks include mineral deposits in the blood vessels and heart that can cause tissues to stiffen. Because of this, for many years there has been a keen interest in better understanding phosphate metabolism in its own right. While PTH is recognized as one of the key regulators of phosphate, there had long been a suspicion among many researchers that PTH alone could not explain all of the intricacies of phosphate metabolism, and that some other factor or factors must be playing a role in this process. In fact, this hypothetical factor was even given a name—"phosphatonin."

FGF-23 and Phosphate Metabolism

Much of current knowledge about calcium and phosphate metabolism grew out of fundamental research conducted over the past 40 years by researchers studying the regulation of cell growth. The cornerstone of this knowledge comes from studies of fibroblasts, cells comprising much of the tissue that provides physical and biochemical support to other tissues. Because they are relatively easy to grow in the laboratory setting, they have long been a favored experimental model for the study of the factors that influence cell growth. In the

early 1970s, NIH-supported researchers showed that small amounts of a mixture of proteins isolated from pituitary glands in the brain could stimulate the growth of fibroblasts in culture. In the mid-1980s, scientists identified two distinct growth-stimulating proteins in this mixture, which they termed "acidic" and "basic" FGFs based on their chemical properties. Nearly 30 years later, over 20 additional members of the FGF family have been identified. They play roles in biological functions as diverse as embryonic development, cell differentiation, nerve cell survival, wound repair, and tumor growth.

The twenty-third member of the FGF family was identified in 2000. FGF-23 is primarily produced by bone cells. It has been shown to have an impact on mineral and salt metabolism. Initial studies of FGF-23's physiological role in humans occurred in the context of several varied rare diseases, including hypophosphatemia (abnormally low levels of phosphate in the blood), rickets (defective mineralization of bones), and osteomalacia (softening of the bones). All of these conditions are characterized by low phosphate levels and bone loss. These diseases were ultimately found to be associated with elevated levels of FGF-23. In one, the FGF-23 protein was normal, but simply overproduced. In another, a mutation in the *FGF-23* gene rendered the protein resistant to degradation. These findings represented some of the first studies to suggest a broad, direct role for FGF-23 in phosphate metabolism and disease, and that unregulated FGF-23 signaling might result in dangerous disruptions in phosphate levels that could have wide-ranging physiologic consequences.

Much of the knowledge about the molecular function of FGF-23 comes from studies conducted in mice in the early 2000s. Either the injection of FGF-23 or the implantation of cells producing high levels of FGF-23

STORY OF DISCOVERY

results in markedly diminished phosphate levels in the blood and elevated levels of phosphate in the urine. Mice genetically engineered to produce high levels of FGF-23 show similar characteristics, as well as more widespread problems such as bone deformation. Conversely, mice engineered to lack the *FGF-23* gene display elevated phosphate levels in the blood and have calcium deposits in many organs, abnormal bone mineralization, and a shortened lifespan. Detailed mechanistic studies in mice supported by the NIH demonstrated that FGF-23 promotes secretion of phosphate by the kidney and increases calcium absorption in the gut. Interestingly, many of these functions are the same as those that had been attributed to the hypothetical “phosphatonin.” While PTH retained an important role in the regulation of calcium and phosphate, it soon became clear that this picture was incomplete, and that FGF-23 was a key player in phosphate regulation.

FGF-23 and Kidney Disease

As FGF-23’s central role in phosphate metabolism was being characterized through studies of animal models and rare human diseases, NIDDK-supported researchers began to extend and expand studies of FGF-23, especially in individuals in whom proper phosphate metabolism was compromised, such as people with CKD. They found that in early stages of kidney disease, FGF-23 levels increase as kidney function declines. In fact, FGF-23 levels begin to rise before clinically significant changes in calcium, phosphate, and PTH are detectable in the blood. It was proposed that a small, initial rise in circulating phosphate levels very early in kidney disease may trigger an increase in FGF-23 as the body prompts the kidneys to excrete the excess phosphate. This increase in FGF-23 levels seems to precede the previously observed increase in PTH levels in CKD patients.

FGF-23 and PTH reduce the activity of phosphate transporters in the kidney, leading to diminished phosphate reabsorption and increased phosphate excretion in the urine. As CKD progresses and circulating phosphate levels rise, FGF-23 levels in the blood gradually increase to try to restore mineral balance. As kidney function further declines, more FGF-23 is produced in response to subsequent increases in serum phosphate concentrations. One NIH-supported study indicated that by the time patients reach kidney failure, FGF-23 levels can be up to 1,000-fold higher than those seen in healthy people.

While increases in FGF-23 levels are associated with a decline in kidney function, it is not clear whether FGF-23 plays a direct, causative role in this progression. Nevertheless, several lines of evidence suggest that measuring circulating levels of FGF-23 in patients with early-stage kidney disease could yield valuable information regarding their prognosis. One study of over 200 people with nondiabetic kidney disease found that increased FGF-23 levels correlated with risk of kidney disease progression, and that this risk was related to the levels of FGF-23 in the blood. Among individuals who are starting dialysis, elevated FGF-23 levels are associated with an increased risk of death both during the first year and over the first 2 years. The ability to identify and stratify patients with CKD based on their initial levels of FGF-23 would provide valuable information to physicians, suggesting that individuals at greater risk of progression due to elevated levels of FGF-23 might benefit from more aggressive care.

Premature death from all causes, and from cardiovascular disease in particular, is higher in people with CKD than in healthy adults. In fact, individuals with CKD are much more likely to die than to survive long enough to progress to kidney failure. Cardiovascular disease is the leading cause of death

STORY OF DISCOVERY

in people with kidney disease, and abnormally high levels of FGF-23 are associated with increased risk of cardiovascular disease in patients with CKD. Research supported by the NIDDK and other NIH components has shown that elevated FGF-23 levels are associated with an enlarged heart, which indicates that this muscle is working harder than it should to pump blood throughout the body. Support for the notion that these changes are a consequence of higher FGF-23 levels comes from NIDDK-supported experiments conducted in animals. Mice that received injections of FGF-23 developed enlarged left ventricles, suggesting that FGF-23 may actually cause this form of cardiovascular disease rather than simply be a byproduct of it.

FGF-23 and Acute Kidney Injury

In contrast to CKD, which usually progresses slowly over time, acute kidney injury (AKI), also called acute renal failure, is characterized by a relatively rapid loss of kidney function, usually over a period of several hours or days. The resulting inability to excrete waste products and maintain fluid and salt balance poses urgent health problems for patients and their physicians. AKI may arise from a number of causes, such as sepsis (a serious, whole-body inflammatory reaction caused by infection), decreased blood pressure, or kidney damage from drugs or other toxins. Even though most people with AKI will regain some degree of kidney function, many do not, and this medical condition is associated with high in-hospital mortality rates. There is no effective drug therapy to reverse AKI. The goal of treatment—which may include dialysis along with other approaches—is to prevent fluid and waste from building up in the body while waiting for the kidneys to resume functioning.

The first suggestion that FGF-23 might play a role in AKI came in 2010. An individual was admitted to the

hospital and diagnosed with AKI. Analysis of his urine showed that his FGF-23 levels were more than six times higher than normal. A subsequent NIH-supported study of 12 people with AKI found significantly higher than normal FGF-23 levels. The degree of the elevation correlated with severity of AKI and those with particularly high FGF-23 levels were more likely to die. Another NIDDK-supported study of 30 people with AKI found similar results. Those with elevated FGF-23 levels were more likely to require dialysis and die. More recently, an NIH-supported analysis of data collected from more than 3,000 people over the age of 65 as part of a study of risk factors for cardiovascular disease found that higher FGF-23 levels were associated with greater risk of hospitalization for AKI over a 10-year period.

For over 40 years, researchers and physicians have thought of AKI as a condition with causes and consequences distinct from those of CKD. However, more recent analyses have suggested that both conditions, rather than being two distinct phenomena, may in fact lie on a continuum. This hypothesis proposes that these two conditions differ not so much in their fundamental nature as they do in the speed with which they emerge. Both are characterized by a loss of kidney function and each is a risk factor for the other: people who have experienced a bout of sudden AKI and who recover are at increased risk of developing CKD in the future; reciprocally, individuals with slow-developing CKD are at increased risk of AKI as their disease progresses. Furthermore, both AKI and CKD place individuals at higher risk of the subsequent development of cardiovascular disease, kidney failure, and premature death. Evidence that FGF-23 might be involved in AKI as well as in CKD, either as a marker for disease severity or prognosis or as an active contributor to the disease process, further strengthens the argument that this factor plays a key role in the maintenance of normal kidney function.

STORY OF DISCOVERY

FGF-23: Biomarker, or More?

In recent years, there has been much enthusiasm regarding the potential benefits of biomarkers, which are molecules that can be easily detected and measured that may be indicators of an underlying condition that is otherwise difficult to evaluate. There seems to be fairly strong evidence that FGF-23 represents, at the very least, a potentially valuable biomarker for kidney disease initiation, prognosis, and progression. If FGF-23 can be validated as a biomarker for kidney disease—either in CKD, AKI, or both—increases in its levels could enable physicians to detect CKD early in the course of the disease using a simple blood test before overt symptoms appear and before irreversible organ damage occurs. Increased levels of FGF-23 could also allow doctors to more accurately assess the prognosis of patients with AKI. Alternatively, FGF-23 levels and trends over time could allow physicians to predict a given person's likely clinical path. Those with more dire prognoses could be treated earlier or receive more aggressive therapy, allowing a more personalized approach to treatment. Indeed, a recent study conducted as part of the NIDDK-supported Chronic Kidney Disease Biomarkers Consortium reviewed the records of more than 13,000 healthy volunteers who enrolled in a clinical study between 1990 and 1992, and found that people with higher levels of FGF-23 when they first entered the study had an increased risk of kidney failure over the

subsequent 20 years. This correlation was seen across all people, regardless of their age, race, and kidney function at the time that they enrolled.

More research is needed to elucidate the ways in which FGF-23 exerts its multiple effects. If FGF-23 can be conclusively linked to specific disease mechanisms as a causative agent in CKD progression or its complications—rather than merely an indicator of it—this protein's impact could be quite profound. Studies in mice suggest that elevated levels of FGF-23 may play a direct role in the development and progression of CKD rather than simply be a by-product of the disease. Pilot studies in humans are currently testing medications to lower circulating levels of FGF-23 and phosphate in patients with moderate CKD in the hopes of developing strategies to lower the risk of complications. Approaches that inhibit FGF-23 action might prevent, lessen, or slow damage to the kidneys and vasculature of patients with CKD. From these studies, new insights into CKD initiation and progression may be found, new drug targets may be identified, and new treatment approaches may be developed.

Scientists have progressed a long way from a time when they felt the need to propose a hypothetical “phosphatonin” to explain the complex regulation of phosphate metabolism, to the current day, when they are characterizing the role of FGF-23 in these key physiological processes.

SCIENTIFIC PRESENTATION

Sex Differences in Kidney Disease

Dr. Sharon Anderson

Dr. Sharon Anderson is the Interim Chair of the Department of Medicine at the Oregon Health and Science University (OHSU). Her research interests include the role of high blood pressure in kidney disease and mechanisms of progression of chronic kidney disease, including diabetic nephropathy, the aging kidney, and polycystic kidney disease. She is an active physician, researcher, and educator.

Dr. Anderson received her M.D. from Louisiana State University Medical Center. After internal medicine residency training at OHSU, she completed her clinical nephrology training at the Beth Israel Deaconess Medical Center and her research training at the Brigham and Women's Hospital, Harvard Medical School. She is a past President of the American Society of Nephrology, and was the first woman to lead the Society when she assumed the position in 2009.

At the September 2014 meeting of the National Diabetes and Digestive and Kidney Diseases Advisory Council, Dr. Anderson reviewed current knowledge regarding the ways in which kidney disease can affect males and females differently, and shared the results of recent studies of these differences.

Males and females differ in anatomy, but there are important physiological differences between the sexes as well. One of the more striking ones is the observation that certain diseases are much less severe in women than in men. Studies have suggested that this may arise, to some degree, as a consequence of a beneficial effect of the female sex hormone estrogen, which is

present in much higher levels in women; or to a negative effect of testosterone, the male sex hormone. Previous data indicate that estrogen may have a protective effect in the eye, brain, bones, and, possibly, the cardiovascular system. However, as recently as 2002, there was very little information about whether estrogen may play a role in kidney disease in humans.

Sex differences in chronic kidney disease have been long recognized. As long ago as the early 1950s, it was known that male rats placed on a high-protein diet developed increasing levels of protein in their urine—a sign of kidney damage—over their lifespan. However, female rats placed on the same diet did not show similar signs of kidney damage. Further experiments suggested that this difference was related, at least in part, to the rats' respective sex hormones. Male rats on the high-protein diet that had been castrated surgically—and thus no longer produced biologically significant levels of the male sex hormone testosterone—had much lower levels of protein in their urine when placed on the high-protein diet. This protective effect was reversed if the castrated rats were given injections of testosterone. Similarly, female rats placed on the high-protein diet that were given testosterone had urine protein levels that were similar to those of normal male rats. This led to the notion that testosterone might have a detrimental effect on kidney health, and/or that estrogen might have a beneficial, protective effect.

Dr. Anderson noted that chronic kidney disease arising from several causes—including IgA nephropathy, membranous glomerulopathy, focal and segmental glomerulosclerosis, polycystic kidney disease, and

SCIENTIFIC PRESENTATION

kidney disease of unknown origin—progresses faster in men than in women. The notable exception to this pattern is seen in women with diabetic kidney disease, whose kidney disease progresses at a rate similar to that seen in men. Why this is so is not clear, but it may relate to the fact that women with this form of kidney disease tend to be older and post-menopausal, and therefore they produce much lower levels of estrogen.

Sex Differences in Animal Models of Polycystic Kidney Disease

Dr. Anderson turned to experiments that she and her colleagues have conducted in a rat model of polycystic kidney disease (PKD). Autosomal dominant PKD (henceforth simply “PKD”) is the most common genetic cause of chronic kidney failure. PKD is characterized by the growth of numerous fluid-filled cysts in the kidneys. Over time, growth of these cysts results in enlarged kidneys in which normal tissue is displaced and kidney function is impaired, sometimes quite severely. The first rat model of PKD was identified in 1989 and characterized in 1994. The Han:SPRD rat line was discovered by happenstance; a spontaneous mutation in a line of rats produced animals that developed cystic kidney disease that closely resembled PKD in humans. This rat model replicates much of the disease’s natural history and has become a valuable model system to study PKD.

In the Han:SPRD rat model, estrogen appears protective against cystic kidney disease, with males developing more and larger cysts than females. Male rats with cystic disease exhibit diminished filtering capacity in their kidneys compared to normal rats, and this can be partly reversed by removal of endogenous testosterone by castration. Males also showed higher blood pressure and poorer values for other markers of kidney function. In contrast, female rats with cystic

disease have filtering capacity that is normal, but show a modest loss of this after their ovaries are removed.

The researchers next turned their efforts to trying to understand whether estrogen-like hormones also exerted a protective effect. Estrogen in the body can be converted to alternate forms that can have different effects on cells. Two such molecules are 2-methoxyestradiol (2-ME) and 2-hydroxyestradiol (2-OHE), which appear to share many of the beneficial effects of estrogen but may be safer for clinical use. These estrogen metabolites have a protective effect on the kidneys in some models, so the scientists were curious as to whether the compounds would slow the growth of cysts and/or preserve kidney function in male rats with cystic kidney disease.

When these two compounds were tested in male Han:SPRD rats, the results were surprising. In the study, the volume of the kidney occupied by cysts was greatest in the untreated male rats and slightly lower in those that had been castrated, as previously shown. Treatment with 2-ME showed a reduction similar to castration, but treatment with 2-OHE reduced cyst volume significantly. Similar results were seen when kidney filtering capacity was measured, with 2-OHE showing a larger beneficial effect than either castration or 2-ME. The mechanism through which this effect is mediated is not known, but these results suggest that some estrogen metabolites may represent a novel, safe intervention to slow progression of PKD in males.

Patients with PKD often develop cysts in their livers, as well. In contrast to what is seen in the kidneys, however, where females have less pronounced cyst growth, the opposite is seen in the liver. The number and size of liver cysts in people with PKD is associated not only with age and severity of kidney cysts but also with female sex and number of pregnancies,

SCIENTIFIC PRESENTATION

suggesting that exposure to estrogen might promote cyst growth in the liver while inhibiting it in the kidneys.

Sex Differences in Acute Kidney Injury

Dr. Anderson next spoke about a possible role for sex hormones in acute kidney injury (AKI). This condition (also called “acute renal failure”) is a serious medical condition characterized by a relatively rapid loss of kidney function, usually over a period of several hours or days. It is a relatively common complication among hospitalized patients. Even though a significant fraction of patients with acute kidney injury will regain kidney function, many do not, and this medical condition is associated with high in-hospital mortality rates among the critically ill. AKI is more common in older patients and non-White patients; rates are also higher in men than in women.

Of all of the cases of AKI during hospitalization, surgical patients undergoing heart surgery are at particular risk. There are multiple animal models to study AKI, but a colleague of Dr. Anderson’s used one that had not previously been used to study this condition. In this model, cardiac arrest is induced in a mouse through the injection of potassium chloride. With the circulation of blood halted, cells and tissues become starved for oxygen and begin to die. Ten minutes later, drugs are administered and chest compressions are begun to restart the heart. In the AKI study using this model, 24 hours after they recovered, the animals’ kidneys were assayed for function and structural damage.

The results suggested that estrogen protected against AKI in mice that underwent cardiac arrest: kidney damage was much less severe in female mice than in males. Further support for this conclusion was seen in experiments in which female mice that had had their ovaries removed showed more severe kidney

damage than those that had not following cardiac arrest, but injection of estrogen to such mice prior to the experiment averted kidney damage. This finding supports the contention that estrogen can protect the kidneys when their supply of oxygen is interrupted.

Mechanism of Estrogen Protection

The effect of estrogen on cells is mediated in general through at least two known estrogen receptors (ER), ER α and ER β . ER α has been linked to rapid cellular responses that do not require gene activation; ER β is more closely associated with responses to vascular and likely other tissue injury that do require gene activation. ER α receptors are the predominant form of the receptor found in the female kidneys, and ER β receptors are the main form in the male kidney. However, the protective effect of estrogen in the cardiac arrest AKI mouse model did not depend on the presence of either of these estrogen receptors, as female mice lacking either the ER α or the ER β genes still were protected from most of the AKI damage by estrogen. Moreover, treatment with a drug that blocks the binding of estrogen to both ERs did not reverse the protective effect of estrogen. Estrogen also did not appear to work through another known receptor, GPR30. These observations suggest that the protective effect of estrogen in kidneys might be mediated by a yet unknown pathway, perhaps through the direct interaction between the hormone and other cellular molecules.

Sex Differences in Biological Research and Implications for Medical Care

Dr. Anderson closed by noting that the vast majority of medical research in the past was conducted in cells or animals that were either male or whose sex was not reported. The recent observations

SCIENTIFIC PRESENTATION

about sex differences in kidney disease underscore the importance of efforts to move toward a more personalized way of practicing medicine. She recommended that future studies should analyze data for differences based not only on sex and gender, but also on age, race/ethnicity, and changes that occur over time—and combinations of these factors. As more is learned, physicians may develop therapeutic approaches for kidney disease based on a patient's individual profile, perhaps choosing early interventions in those at risk of developing disease; or selecting more aggressive treatments in those at risk of rapid progression; and tailoring the treatment to the patient's unique genetic, environmental, and behavioral situation. Using this approach, researchers and doctors will be able to do a better job of deploying the tools they have today while developing better treatments for tomorrow.

On May 14, 2014, Janine A. Clayton, M.D., Director of the NIH Office of Research on Women's Health (ORWH) and Francis S. Collins, M.D., Ph.D., Director of the National Institutes of Health, published a commentary in the journal Nature entitled "Policy: NIH to balance sex in cell and animal studies." Drs. Clayton and Collins announced that the NIH plans to address the issue of sex and gender inclusion across biomedical research through oversight, review, and policy, as well as through collaboration with various stakeholders. For details and updates about this issue and NIH efforts, please see the ORWH webpage "Studying Sex to Strengthen Science (S4)" at <http://orwh.od.nih.gov/sexinscience/>

PATIENT PROFILE

For Capt. John “Jack” Sautter, An Unexpected Diagnosis Becomes a Call to Action *After Discovering That He Had Polycystic Kidney Disease, a Marine Seeks a Better Future for Himself and Others*



Jack Sautter and daughter Katherine

There are many ways to describe John Sautter. First of all, call him Jack. Jack is a Captain in the U.S. Marines and a third generation veteran. He is a lawyer, working as a prosecutor in the Marine Corps. He holds a Ph.D. in political science. He is also a proud son, brother, husband, and father. Until a little over 7 years ago, Jack was also the picture of perfect health, or so he thought.

In one day, though, his life changed forever; he was diagnosed with polycystic kidney disease (PKD), a disease that has been traveling in his family for at least four generations. Rather than simply accept the diagnosis as an insurmountable obstacle, Jack approached this new challenge the same way he had approached every other one he'd faced: he searched

for ways to address it. One path he chose was to volunteer for an NIDDK-supported clinical trial of treatment options for people with PKD and, in doing so, try to build a better life not only for himself but for all people—both now and in the future—with the disease.

Diagnosis

On a crisp fall day in 2007, a then 29-year old Jack enjoyed a spirited rugby game with some friends from law school, followed by a backyard barbeque. He returned home, tired from the evening's activities, and tumbled into bed. When he woke the next morning, his foot was in so much pain that he could barely walk. "I thought I'd broken my foot," playing rugby the previous day, he says. He wasn't particularly worried, though, as he'd injured the same foot playing football years earlier. Still, he thought it best to have it checked out and headed off to the local hospital's emergency room.

"I thought I'd broken my foot," playing rugby the previous day, Jack says.... "I did not go to the hospital thinking that I was going to be diagnosed with PKD."

After a brief physical exam and evaluation, the pain in Jack's foot was diagnosed as gout, which surprised him. Gout is caused by the deposition of crystals

PATIENT PROFILE

of uric acid in the joints—usually in the extremities, commonly in the toes—which causes inflammation and sometimes quite severe pain. These crystals form as a consequence of elevated levels of uric acid in the blood. Usually, uric acid remains dissolved in the blood and is filtered and excreted by the kidneys. High levels of circulating uric acid are sometimes, but not always, an indication of underlying kidney problems.

As the emergency room staff asked questions about Jack's medical history, he told them that several members of his family, including his father, had been confirmed to have PKD, and several other relatives were thought to have had it. "The doctors rolled in an ultrasound machine and held it up to my kidneys...and I could see the cysts, right there." This unexpected news was traumatic and scary, and his initial reaction was one of "panic and fear." He adds, "I did *not* go to the hospital thinking that I was going to be diagnosed with PKD." In that instant, Jack went from a self-described "carefree, young, rugby-playing Marine officer who could do anything, accomplish anything" to someone whose "life was inexorably changed."

There was another reason why this diagnosis was particularly unsettling for Jack. Cardiovascular disease is often seen in people who have longstanding PKD, and it had been just 3 years since his father had passed away after a heart attack. He was 56 years old when he died.

Polycystic Kidney Disease

PKD is a genetic disorder characterized by the growth of numerous fluid-filled cysts in the kidneys. There are two main forms of PKD. The most common is autosomal dominant PKD, which is the kind in Jack's family. Symptoms usually develop between the ages of 30 and 40, but they can begin earlier, even

in childhood. In the United States, about 600,000¹ people were estimated to have PKD in 2000, and cystic disease is the fourth leading cause of kidney failure.

In most cases of autosomal dominant PKD (henceforth referred to simply as "PKD"), the slow progression of cyst growth can go unnoticed for many years. Many people with early-stage PKD have no symptoms, and their physical condition appears normal. The cysts, which can number in the thousands, can profoundly enlarge the kidneys while replacing much of their normal structure, resulting in reduced kidney function and potentially leading to kidney failure and a host of other health problems. Jack says that, when his father was in his 50s, his kidneys were estimated to be the size of small footballs. "You could actually see his kidneys bulging out of his sides," he adds.

Many people with PKD experience a decline in their kidney function as the cysts grow, and about one-half of them progress to kidney failure and require dialysis to live. High blood pressure is another common health problem for people with PKD. In most people with PKD, high blood pressure appears by age 20 or 30; it can lead to serious cardiovascular complications such as heart attack or stroke, both of which contributed to Jack's father's death. Other complications of PKD include urinary tract infections, blood in the urine, and kidney stones.

Jack Sautter Takes Action

Fortunately for Jack, tests at the hospital revealed that his kidney function was normal, suggesting that his PKD had been detected before serious damage had occurred. After the shock of his diagnosis had passed, "I started doing what everybody does in the modern age: I jumped onto the Internet and started reading everything I could." He scoured the websites

PATIENT PROFILE

of hospitals, news organizations, and even blogs in an effort to learn as much as he could about the disease.

In November of 2007, Jack found a set of slides on the website of Tufts University School of Medicine. The presentation was from a conference that the school had hosted on PKD; these particular slides talked about a clinical trial called HALT-PKD. He thought, “Wow, this is really fascinating!” At the time, Jack was attending law school in Vermont; Tufts is located in nearby Boston, Massachusetts. When he discovered that Tufts was one of the sites participating in the trial, he located contact information for the university’s HALT-PKD site and picked up the phone. Shortly after that conversation, he had enrolled in the study.

The HALT PKD Trial

Launched in 2002, the HALT-PKD trial enrolled two groups of volunteers based on their kidney function: those with relatively healthy kidneys were enrolled into “Study A,” and those with more advanced disease and diminished kidney function were enrolled into “Study B.” Jack entered “Study A,” which recruited 558 volunteers with early-stage PKD at seven medical centers around the country. This study tested whether drugs that target the renin-angiotensin system—an important regulator of blood pressure and fluid balance—could slow the progression of the disease.

In the HALT-PKD Study A, the volunteers were given oral medications aimed at lowering their blood pressure, which is expressed as a higher number “over” a lower number. Half were assigned to a group with the goal of achieving “standard” blood pressure, defined as between 120 to 130 over 70 to 80. The other half targeted a lower blood pressure, between 95 to 110 over 60 to 75; while lower than the “standard” target, this is still within the normal range. Within each

group, the participants were started on either a single drug or two drugs to reach their goal. (Those receiving a single drug received a placebo pill as their second “medication.”) Jack was randomly assigned to the low blood pressure group. “I was hoping to be assigned to this group,” he says. “I thought that perhaps I could benefit from the study by having lower blood pressure,” although at the time he signed up he had no way of knowing what the outcome of the trial might be.

Jack was an enthusiastic volunteer in the HALT-PKD study. Even a 7-month deployment to Helmand province in southern Afghanistan from October 2011 to May 2012 could not prevent him from continuing to participate.

Throughout the trial, Jack and his fellow study participants had regular check-ups, provided blood samples every 6 months, and underwent magnetic resonance imaging (MRI) of their kidneys to monitor cyst growth several times. Jack was an enthusiastic volunteer. Even a 7-month deployment to Helmand province in southern Afghanistan from October 2011 to May 2012 could not prevent him from continuing to participate. He asked his physician in the United States to forward his medical records to the unit’s battalion surgeon, who arranged for Jack to visit the local combat hospital. There, he was able to provide blood samples that were analyzed on site; the results were forwarded to the HALT-PKD investigators at Tufts. When Jack returned home, he continued his participation in HALT-PKD until the trial’s end in 2014.

Data collection in HALT-PKD ended in the late summer of 2014. The investigators spent several months analyzing the data, and the results of the trial were

PATIENT PROFILE

announced at the annual scientific meeting of the American Society of Nephrology in November 2014 (see below for more information). Jack is “really looking forward” to learning the results, because they may benefit not only him personally, but also his family members and the larger numbers of people with PKD.

PKD and the Sautter Family: Past, Present, and Future

PKD has a long history in the Sautter family. Jack’s great-grandmother, a member of the first generation of the family to be born in this country, is thought to have had PKD, although she was never formally diagnosed. His grandfather, an Army pilot in World War II, had PKD. His father, a 20-year Army veteran, had it. Jack is acutely aware of the toll exacted by advanced PKD, as he had helped care for his father during the last years of his life. Speaking of him, Jack says, “A lot of things that slowed him down and made life more difficult for him were related to PKD.” These experiences made Jack acutely aware that PKD affects not only the people with the disease themselves, but their families and caregivers as well.

After a tour in Okinawa, Japan and Hawaii, Jack is currently stationed at Camp Pendleton in California, where he lives with his wife and 2-year-old daughter. When he reflects on his participation in the HALT-PKD trial, he knows that, even if the findings from the trial do

not directly help him in the near term, his participation in this research might help other people with PKD in the future. While he says that one motivation for entering the trial was that it might benefit him, he is quick to add, “Like in all things, our decisions are not as simple as just one motive; there are lots of motives.... It wouldn’t shock me if my daughter has PKD.” And, while he hopes that she stays healthy, if she is someday diagnosed, “hopefully, she will benefit from this research.” He adds, “That was definitely on my mind when I was trying to become a part of HALT-PKD and continuing to do the study.”

The NIDDK and the PKD Foundation sponsored the HALT-PKD trials, which consisted of two treatment trials for autosomal dominant polycystic kidney disease. HALT-PKD was the largest and longest study of treatments for this condition. The results were initially reported at the annual scientific meeting of the American Society of Nephrology in November 2014, and were published as this document was going to press. More information about the study outcomes is at www.nih.gov/news/health/nov2014/niddk-17.htm

¹ Grantham JJ, Nair V, Winklhofer F. Cystic diseases of the kidney. In: Brenner BM, ed. *Brenner & Rector’s The Kidney*. Vol. 2. 6th ed. Philadelphia: WB Saunders Company; 2000: 1699-1730.

